Budagovsky 9 Rootstock: Uncovering a Novel Resistance to Fire Blight

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Abstract

Budagovsky 9 (B.9) apple rootstock, displayed a high level of susceptibility (similar to M.9 rootstock) to fire blight bacteria (*Erwinia amylovora)* when leaves of non-grafted B.9 plants were inoculated. However, when older B.9 rootstock tissue was inoculated directly with *E. amylovora*, rootstock tissue was resistant in both grafted and non-grafted plants. Comparisons of four scion cultivar ('Gala', 'Red Yorking', 'Jonagold' and 'Gingergold') and two sources of B.9 rootstock (Oregon, USA and Netherlands) showed no influence of scion cultivar or source of B.9 rootstock on symptom development. Further experiments investigating tissue age and its effect on resistance suggest B.9 asserts a form of persistent age related resistance in response to *E. amylovora*.

INTRODUCTION

New advances in high-density apple production systems have dramatically increased fruit production and quality. Dwarfing rootstocks are necessary in high density orchards to maintain tree size and optimize production, resulting in increased grower dependency on the popular dwarfing rootstock Malling 9 (M.9). However, the incidence of the rootstock phase of fire blight (rootstock blight) has increased due to reliance on susceptible dwarfing rootstocks such as M.9 in combination with susceptible scion cultivars in high-density apple orchard systems. Previous studies have reported tree losses over 50% when trees grafted on M.9 suffer severe rootstock blight infection (Ferree et al., 2002).

Rootstock blight is an untreatable and typically fatal infection of the apple rootstock that results in tree death (Momol et al., 1998; Norelli et al., 2003). Bacteria gain entry into the rootstock through wounds or by the systemic movement of bacteria from scion infections into the rootstock (Momol et al., 1998). Due to the nature of the disease, control is virtually impossible, save for the complete elimination of fire blight from an orchard. Losses are compounded by the high cost of replanting and the loss of orchard productivity for several years (Momol et al., 1999). The only proven method for controlling rootstock bight is the use of fire blight resistant rootstocks at planting (Norelli et al., 2003).

In horticultural trials the dwarfing rootstock Budagovsky 9 (B.9) is comparable to certain strains of M.9 with regard to both tree size control and productivity (Marini et al., 2006). Historically, B.9 has been listed as susceptible to fire blight preventing recommendation as an alternative to M.9 (Lespinasse and Aldwinckle, 2000). However, in recent field trials using grafted trees B.9 has displayed a high degree of resistance to rootstock blight leading to renewed interest in B.9 for future production (Russo et al., 2007). Prior to recommending B.9 for high-density orchards it was imperative to explain the inconsistencies observed in rootstock resistance. Previous research has demonstrated that *E. amylovora* can migrate and be detected in B.9 rootstocks at low populations; indicating bacterial migration is not a limiting factor in rootstock blight severity (Russo et al., 2008).

The objectives of the current study were to determine if bacteria, when directly applied to wounds in the rootstock, influenced B.9 resistance to rootstock blight and to determine if grafted and non-grafted rootstocks behave similarly to bacterial inoculation.

MATERIALS AND METHODS

In the first study, non-grafted M.9, Geneva®16 and B.9 rootstocks grown in the greenhouse were evaluated for resistance to *E. amylovora* according to Norelli et al. (1984). Shoots were inoculated by transversely bisecting the two youngest leaves with scissors dipped in a suspension of *E. amylovora* strain E4001a in potassium phosphate buffer (0.05 M) at 1×10^7 cfu/ml. Lesion length was recorded as a percent of the current year's shoot growth when lesions ceased progressing and used as a measure of susceptibility (Norelli et al., 1984).

In the second study, B.9 rootstocks sourced from the Netherlands (B.9-NE) or sourced from Oregon, USA (B.9-OR) were evaluated in the field for resistance to wound inoculation of older rootstock tissue by *E. amylovora* in 2005 and 2006. Four- and five-year-old grafted apple trees with one of four scion varieties ('Gala', 'Red Yorking', 'Jonagold' and 'Gingergold') on either M.9, G.16 or the two B.9 strains and non-grafted M.9 and G.16, and the two B.9 strains were wounded approximately 5 cm deep with a drill at 5 to 10 cm below the graft union for grafted trees and 10 cm above the soil line for non-grafted rootstocks. Wounds were inoculated with 100 µl of *E. amylovora* strain E4001a at three inoculum concentrations, 10^5, 10^7 and 10^9 cfu/ml in 2005, and 10^7 and 10^9 cfu/ml in 2006. Control plants were wounded and treated with potassium phosphate buffer (0.05 M). Development of typical rootstock blight lesions was based on the appearance of diagnostic symptoms; including the production of bacterial ooze or the appearance of a fire blight canker. Effects of rootstock and inoculum concentration on the development a rootstock blight lesion were evaluated using logistic regression.

RESULTS AND DISCUSSION

When shoots of non-grafted rootstocks were inoculated in the greenhouse with *E. amylovora,* results supported previous resistance evaluations in which B.9 rootstock was found to be susceptible to fire blight (Fig. 1). Leaf inoculated, B.9 and M.9 rootstocks became severely infected with nearly 100% of the current shoot length blighted. In contrast, Geneva®16 a highly resistant rootstock did not develop shoot bight or other necrosis associated with fire blight infection.

In the wound inoculation experiment, neither scion cultivar nor inoculum concentration had a significant effect on fire blight sensitivity. Results therefore represent the proportion of symptomatic rootstocks combined by rootstock (Fig. 2). Contrary to results when B.9 was leaf inoculated, wound inoculated B.9 rootstocks were found to be highly resistant to infection. Both clones of B.9 rootstock were indistinguishable from the resistant rootstock Geneva®16 with regard to symptom development, which ranged from 20 to 25%. It is important to note that symptom development was not indicative of tree death. Geneva®16 and B.9 rootstock symptoms, including canker formation and ooze, were observed but were not associated with adverse effects on tree health. M.9 rootstock retained its susceptible phenotype, with over 70% of M.9 rootstocks developing typical rootstock blight symptoms. Symptom development in M.9 rootstocks, unlike in B.9 and Geneva®16, was highly correlated with tree/rootstock mortality. These findings were consistent for both grafted and non-grafted rootstocks, indicating that grafting was not a critical factor in resistance.

Although the evaluation of leaf tissue is often used as the conclusive measure of rootstock susceptibility results obtained, when wounds made in rootstock tissue were directly inoculated, offer a new perspective on B.9 sensitivity to fire blight. Based on these results we concluded B.9 tissue exhibits differentially expressed resistance. *Malus* spp. have been shown to exhibit varying degrees of resistance in breeding evaluations, but a complete reversal of susceptibility has not been previously described (van der Zwet and Miller, 1987). B.9 resistance appears to be associated with tissue type: succulent tissue is susceptible whereas woody tissue is resistant. This form of resistance could prove beneficial to rootstock breeders since rootstocks produce little to no succulent tissue in the field.

CONCLUSIONS

The current study provides evidence for a high level of fire blight resistance in woody B.9 tissue. B.9's novel resistance is likely contingent on tissue development. The categorization of B.9 rootstock as susceptible was primarily based on the evaluation of susceptible leaf tissue. Although imprecise, it is unlikely that breeders will abandon the high throughput nature of leaf inoculation in favor of more intensive evaluations. This illuminates the need for improved genetic understanding of fire blight resistance to facilitate the breeding and evaluation of new cultivars.

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Figures

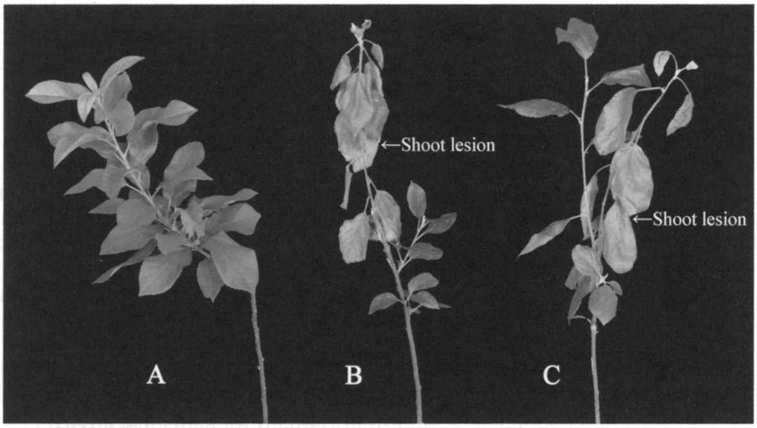

Fig. 1. Susceptibility of 3 non-grafted apple rootstocks to leaf inoculation with *E. amylovora*, A) Geneva® 16 (resistant), B) M.9 (susceptible), and C) B.9 (susceptible). Rootstocks were inoculated by transversely bisecting the two youngest leaves with scissors dipped in *E. amylovora* strain E4001a at 1×10^7 cfu/ml.

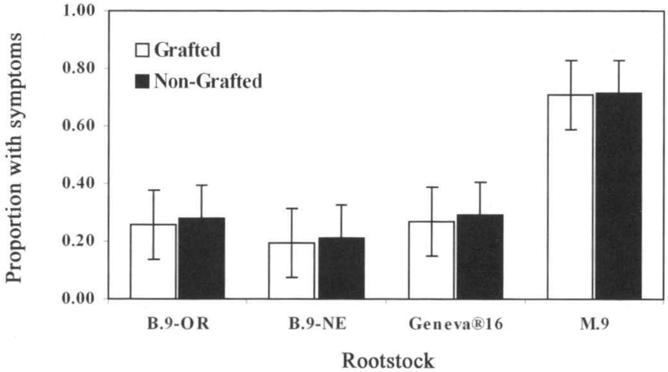

Fig. 2. Proportion of B.9 from Oregon and Netherlands sources, Geneva® 16 and M.9 rootstocks that developed typical rootstock blight symptoms when drill inoculated with *Erwinia amylovora*. Graph represents combined data for 4 varieties ('Gala', 'Red Yorking', 'Jonagold' and 'Gingergold') and 4 inoculation concentrations (10^5, 10^7 and 10^9 cfu/ml) from 2005 and 2006 experiments.